# EFFECT OF CHRONIC APPLICATION OF QUERCETIN AND ACUTE DOSE OF T-2 TOXIN ON HAEMATOLOGICAL PARAMETERS OF RABBITS

## VPLYV CHRONICKEJ APLIKÁCIE KVERCETÍNU A AKÚTNEJ DÁVKY T-2 TOXÍNU NA HEMATOLOGICKÉ PARAMETRE KRÁLIKA

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## ABSTRACT

The aim of the present study was to determine effect of chronic application of quercetin in various doses and acute dose of T-2 toxin on selected haematological parameters of rabbit's blood. Animals were divided into two control groups (C1 and C2) and experimental groups (E1 – E6). Experimental groups received quercetin in injectable form at 10 µg.kg$^{-1}$ in E1 and E2 group, 100 µg.kg$^{-1}$ in E3 and E4 group and 1000 µg.kg$^{-1}$ in E5 and E6 group without T-2 toxin for 90 days. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria) to C2, E2, E4 and E6 group at dose 0.08mg per kgof body weight 72 hours before slaughter was applied. Significant increase ($P$< 0.05) of MI% (medium-size cell percentage) in E3 (100 µg.kg$^{-1}$ of quercetin), E4 (100 µg.kg$^{-1}$ of quercetin and 0.08 mg.kg$^{-1}$ of body weight) and E5 (1000 µg.kg$^{-1}$ of quercetin) groups in comparison with the control group (C1) was observed. Significant increase of MI% in E2 (10 µg.kg$^{-1}$ of quercetin and 0.08 mg.kg$^{-1}$ of body weight), E3, E4, E5 and E6 (1000 µg.kg$^{-1}$ of quercetin and 0.08 mg.kg$^{-1}$ of body weight) in comparison with E1 (10 µg.kg$^{-1}$ of quercetin) was observed. Also we observed significant ($P$< 0.05) increase of MI% in E3 group *vs.* control group (C2).

Higher values in WBC (total white blood cell count), MID (medium-size cell count) and LYM (lymphocytes count) in experimental groups and insignificant decrease of LY% (lymphocyte percentage) and GRA% (granulocytes percentage) in comparison with the control group was found. Other haematological parameters were not influenced by this natural antioxidant and T-2 toxin.

**Key words:** quercetin, T-2 toxin, haematological parameters, rabbit

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## INTRODUCTION

Quercetin is a polyphenolic flavonoid, classified as a flavonol. Flavonoids seem to play an important role in human health and to possess beneficial effects in the prevention of human diseases. The antioxidant capacity of these molecules seems to be responsible for many of their beneficial effects and confers a therapeutic potential in diseases such as cardiovascular diseases, gastric or duodenal ulcers, cancer and hepatic pathologies (González Gallego et al., 2007). Quercetin is highly abundant in food and beverage sources that are part of the human diet such as broccoli, lettuce, apples, tomatoes, onions, tea and coffee (Jellin et al., 2003). Within the flavonoid family, quercetin is the most potent scavenger of ROS (Heijnen et al., 2002) and possesses strong anti-inflammatory capacities as well (Orsolic et al., 2004).

Mycotoxins are natural and very stable toxins, with relatively low-molecular weight secondary metabolites of fungal origin, which can contaminate a large variety of feed mixtures (Labuda et al., 2009; Tančinová and Labuda, 2009) grains and foodstuffs worldwide, a variety of foods and beverages, including both plant-based products and animal products (Schollenberger et al., 2007; Ranzenigo et al., 2008).

Trichotecenemycotoxins are very large family of chemically related toxins produced by various species of *Fusarium, Myrotecium, Trichoderma*, *Cephalosporiu*, etc.(Wannemacher and Neufeld, 1991). T-2 toxin is some of the most important and toxic trichothecenemycotoxinoccurring in various agriculture products (Iwahashi et al. 2008). Lipophilic nature of T-2 toxin suggests that they are easily absorbed through skin, gut, and pulmonary mucosa (Bunner and Morris, 1988). Trichotecene causes multiorgan effect including emesis, and diarrhea, weight loss, nervous disorders, cardiovascular alterations, immunodepression, hemostaticderangements, skin toxicity, and bone marrow damage (Wannemacher and Neufeld, 1991).

The aim of the present work was to determine effect of chronic application of quercetin in various doses and acute dose of T-2 toxin on selected haematological parameters of rabbit's blood.

## MATERIALSANDMETHOD

### *Animals and diet*

Adult female rabbits (n = 20) and male rabbits (n = 20) of meat line M91, maternal albinotic line (crossbreed Newzealand white, Buskat rabbit, French silver) and paternal acromalictic line (crossbreed Nitra's rabbit, Californian rabbit, Big light silver) were used in experiment. Rabbits were healthy and their condition was judged as good at the commencement of the experiment. Water was available at any time from automatic drinking troughs. Groups of adult animals were

balanced for age (150 days) and body weight (4 ± 0.5 kg) at the beginning of the experiment. Adult rabbits were fed diet of a 12.35 $MJ.kg^{-1}$ of metabolizable diet (Table 1) composed of a pelleted concentrate.

Animals were divided into two control groups (C1 and C2) and experimental groups (E1 – E6). Experimental groups received quercetin in injectable form at 10 $\mu g.kg^{-1}$in E1 and E2 group, 100 $\mu g.kg^{-1}$in E3 and E4 group and1000 $\mu g.kg^{-1}mg.kg^{-1}$ in E5 and E6 groupgroupwithout T-2 toxin for 90 days. T-2 toxin (Romer Labs Division Holding GmbH, Tulln, Austria)to C2, E2, E4 and E6 groupat dose 0.08 mg per kg of body weight 72 hours before slaughter was applied.

In this animal study, institutional and national guidelines for the care and use of animals were followed, and all experimental procedures involving animals were approved by the State Veterinary and Food Institute of Slovak Republic, no. SK CH 29004.

*Table 1Applicationof quercetin and T-2 toxin in porcinebloodin vitro.*

| Group | Quercetin ($\mu g.kg^{-1}$) | T-2 toxin ($mg.kg^{-1}$ of body weight, 72 hours before slaughter) |
|---|---|---|
| C1 | 0 | 0 |
| C2 | 0 | 0,08 |
| E1 | 10 | 0 |
| E2 | 10 | 0,08 |
| E3 | 100 | 0 |
| E4 | 100 | 0,08 |
| E5 | 1000 | 0 |
| E6 | 1000 | 0,08 |

C – control group, E1 – E6 – experimental groups with various doses of quercetin alone or in combination with T-2 toxin.

### *Bloodsampling and analyses*

Blood samples from *vena auricularis* were taken from all animals by macromethods in morning at the end of experiment.

In whole blood, selected haematological parameters [total white blood cell count (WBC), lymphocytes count (LYM), mediumsize cell count (MID), granulocytes count (GRA), lymphocyte percentage (LYM%), mediumsize cell percentage (MID%), granulocytes percentage (GRA%), red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), red cell distribution width (RDWc), platelet count (PLT), platelet percentage (PCT), mean platelet volume (MPV) and platelet distribution width (PDWc)] were measured using haematology analyzer Abacus junior VET (Diatron®, Vienna, Austria).

### *Statistical analyses*

The data used for statistical analyses represent means of values obtained in blood collection (end of the experiment). To compare the results, one-way ANOVA test was applied to calculate basic statistic characteristics and to determine significant differences amongthe experimental and control groups. Statistical software SIGMA PLOT 11.0 (Jandel, Corte Madera, CA, USA) was used. Differences were compared for statistical significance at the level $P< 0.05$.

*Table 2 Chemical composition (g.kg$^{-1}$) of the experimental diet.*

| Component | |
|---|---|
| Dry matter | 926.26 |
| Crude protein | 192.06 |
| Fat | 36.08 |
| Fibre | 135.79 |
| Non-nitrogen compounds | 483.56 |
| Ash | 78.78 |
| Organic matter | 847.49 |
| Calcium | 9.73 |
| Phosphorus | 6.84 |
| Magnesium | 2.77 |
| Sodium | 1.81 |
| Potassium | 10.94 |
| Metabolizable energy | 12.35 MJ.kg$^{-1}$ |

## RESULTS AND DISCUSSION

The results are presented in Table 3. Quercetin had no influence on the most observed parameters ($P> 0.05$). Significant increase ($P< 0.05$) of MI% in E3 (100 µg.kg$^{-1}$ of quercetin), E4 (100 µg.kg$^{-1}$ of quercetin and 0.08 mg.kg$^{-1}$ of body weight) and E5(1000 µg.kg$^{-1}$ of quercetin) groups in comparison with the control group(C1) was observed.Significant increase of MI% inE2 (10 µg.kg$^{-1}$ of quercetin and 0.08 mg.kg$^{-1}$ of body weight), E3, E4, E5 and E6 (1000 µg.kg$^{-1}$ of quercetin and 0.08 mg.kg$^{-1}$ of body weight) in comparison with E1 (10 µg.kg$^{-1}$ of quercetin) was observed.Also we observed significant ($P< 0.05$) increaseof MI% in E3group *vs.*control group (C2).Quercetin at the lowest dose had no effect on observed parameters and the values from this group were similar to the control. Higher doses of quercetin caused significant increase of MI%. Treatment of T-2 toxin also increased MI%. Parabathina et al. (2011) found the similar results in study with rutin and quercetin on rabbits. Authors observed increase in eosinophils after 28 days of treatment. As eosinophils are part of MI%, thus the increase of MI% in our study could be the result of increase count of eosinophils by quercetin treatment. The mechanism of action is not clear yet.

Slight increase of MI% was found in experiment with pesticide bendiocarbamate applied to rabbits (Capcarova et al., 2010). In our experiment T2 toxin caused also increase of MI% as pesticide. Probably toxic substances provoke some parts of leukocytes as monocytes and eosinophil to respond on the exposure by multiplying of their number.

Application of T-2 toxin insignificantly (*P*> 0.05) increased WBC in E1 and E3 groups in comparison with the control group.Increase of WBC is normal reaction to foreign substances, which alter their normal physiological processes (Adebayo et al. 2010; Atta et al., 2010). On the contrary, Gentry and Cooper (1981) found that intravenous administration of T-2 toxin significantly decreased WBC in rabbits. In another study Capcarova et al. (2010) found significant decrease of WBC after bendiocarbamateadditions in rabbits. In this cause it seems that the dose and the length of exposure played the main role of WBC responds. High doses of toxic substances cause decrease of WBC and low doses could raise the WBC parameter. In our study the dose of T2 toxin was low and close to natural exposure of T2 toxin in rabbits feed.

Quercetin/T-2 toxin preparation caused slight increase (P > 0.05) in LYM in all experimental groups in comparison with the control group.Daily consumption of 60 mg isoflavonesimmediate one yeardid not cause any changes in LYM in women(Soung et al., 2006). Dönmez et al. (2012) found that aflatoxin at doses 250 µg per day significantly (*P*< 0.05) decrease LY% in merino rams. In our experiment T2 toxin decreased LY%, however without significant confirmation (P>0.05).

Pang et al. (1988) found that exposure of T-2 toxin in pigs caused some alterations in lymphocyte and neutrophils counts. The similar results were observed by Nikulin et al. (1997) in study with strains that produce mycotoxins*Stachybotrysatra* on mice. Our results did not confirm alterations in lymphocytes and neutrophils counts after T2 toxin exposure. The discrepancies in the results are due to using of different dose of mycotoxin, different kinds of animal subjects and length of treatment periods.

In this study we observed insignificant (*P*> 0.05) decrease inGRA% in all experimental groups *vs.*the control group.In another studyCapcarova et al. (2011) found the similar results with sumac (*Rhuscoriaria*)inclusion to the feed for rabbits.

The values of other haematological parameters (GRA,HGB, MCV, MCH, MCHC, RDWc, PLT, PCT and MPV) were not influenced (*P*> 0.05) after quercetin/T-2 toxin treatment.We have not found other evidence in literature about the effect of quercetin/T-2 toxin on haematological parameters of rabbits.

**Table 3Hematologicalparametersofrabbitsafter quercetin treatment and T-2 toxinapplication.**

| Parameter | C | C2 | E1 | E2 | E3 | E4 | E5 | E6 |
|---|---|---|---|---|---|---|---|---|
| WBC | 12.77 ± 2.93 | 9.85 ± 1.19 | 9.71 ± 0.73 | 10.30 ± 1.83 | 9.65 ± 1.37 | 10.01 ± 1.06 | 8.06 ± 0.47 | 9.64 ± 1.96 |
| LYM | 3.99 ± 0.44 | 4.66 ± 1.80 | 4.30 ± 1.57 | 5.66 ± 1.76 | 4.78 ± 1.04 | 6.29 ± 0.39 | 4.16 ± 1.53 | 5.53 ± 1.44 |
| MID | 0.83 ± 0.67 | 0.50 ± 0.13 | 0.12 ± 0.13 | 0.46 ± 0.25 | 1.14 ± 0.49 | 0.63 ± 0.31 | 0.76 ± 0.16 | 0.69 ± 0.32 |
| GRA | 5.99 ± 1.56 | 6.07 ± 1.65 | 5.13 ± 0.93 | 4.87 ± 1.66 | 4.21 ± 1.44 | 2.76 ± 0.42 | 5.05 ± 1.48 | 5.27 ± 1.00 |
| LY% | 43.32 ± 16.61 | 57.26 ± 24.59 | 38.90 ± 16.05 | 48.56 ± 16.43 | 51.72 ± 10.13 | 52.03 ± 19.00 | 50.06 ± 11.99 | 53.76 ± 14.07 |
| MI% | 2.20 ± 1.17$^{a}$ | 5.03 ± 1.01$^{b}$ | 1.32 ± 1.18$^{c}$ | 6.16 ± 1.04$^{c}$ | 10.57 ± 2.33$^{abc}$ | 8.13 ± 1.94$^{ac}$ | 8.75 ± 1.96$^{ac}$ | 7.00 ± 2.69$^{c}$ |
| GR% | 59.42 ± 26.53 | 58.13 ± 11.00 | 58.52 ± 17.50 | 47.46 ± 13.90 | 39.66 ± 12.42 | 39.80 ± 17.84 | 53.32 ± 8.42 | 50.42 ± 20.04 |
| RBC | 6.19 ± 0.67 | 7.00 ± 0.49 | 6.51 ± 0.54 | 6.98 ± 0.39 | 6.35 ± 1.05 | 6.50 ± 1.01 | 6.72 ± 0.30 | 7.08 ± 0.40 |
| HGB | 130.00 ± 3.55 | 132.50 ± 2.64 | 127.80 ± 9.73 | 134.40 ± 7.43 | 130.50 ± 3.78 | 122.66 ± 17.24 | 130.83 ± 5.56 | 132.00 ± 4.76 |
| HCT | 37.85 ± 6.05 | 41.83 ± 2.80 | 39.89 ± 2.13 | 41.44 ± 2.15 | 37.96 ± 6.45 | 38.12 ± 4.63 | 40.74 ± 1.13 | 41.72 ± 1.89 |
| MCV | 60.80 ± 4.76 | 60.00 ± 1.63 | 61.40 ± 3.20 | 59.60 ± 3.28 | 59,60 ± 1.67 | 59.00 ± 3.00 | 60.66 ± 1.96 | 58.75 ± 3.86 |
| MCH | 19.76 ± 0.79 | 19.00 ± 0.94 | 19.64 ± 0.48 | 19.26 ± 0.65 | 19.24 ± 0.52 | 18.96 ± 1.01 | 19.43 ± 0.63 | 18.60 ± 0.71 |
| MCHC | 325.60 ± 13.50 | 318.25 ± 14.15 | 317.25 ± 10.78 | 324.40 ± 11.54 | 322.40 ± 7.30 | 321.66 ± 7.23 | 320.83 ± 8.23 | 316.00 ± 9.41 |
| RDWc | 15.96 ± 1.94 | 15.27 ± 0.78 | 14.86 ± 0.16 | 15.24 ± 0.54 | 16.90 ± 1.96 | 15.56 ± 1.33 | 15.60 ± 0.98 | 16.07 ± 0.75 |
| PLT | 379.25 ± 91.41 | 298.00 ± 84.71 | 238.20 ± 26.67 | 248.60 ± 19.32 | 297.00 ± 28.77 | 318.00 ± 8.48 | 282 ± 79.85 | 333.75 ± 78.52 |
| PCT | 0.18 ± 0.08 | 0.17 ± 0.05 | 0.13 ± 0.01 | 0.14 ± 0.01 | 0.20 ± 0.08 | 0.19 ± 0.01 | 0.16 ± 0.04 | 0.19 ± 0.06 |
| MPV | 5.66 ± 0.27 | 5.80 ± 0.29 | 5.70 ± 0.70 | 5.92 ± 0.37 | 5.64 ± 0.24 | 5.95 ± 0.35 | 5.86 ± 0.23 | 5.80 ± 0.34 |
| PDWc | 30.36 ± 1.11 | 30.92 ± 1.22 | 29.66 ± 2.49 | 31.12 ± 1.27 | 29.94 ± 1.11 | 31.70 ± 0.70 | 30.46 ± 0.78 | 30.85 ± 1.82 |

WBC, total white blood cell count ($10^9$/l); LYM, lymphocytes count ($10^9$/l); MID, medium-size cell count; GRA, granulocytes count ($10^9$/l); LY%, lymphocyte percentage; MI%, medium-size cell percentage; GRA%, granulocytes percentage; RBC, red blood cell count ($10^{12}$/l); HGB, haemoglobin (g/l); HCT, haematocrit (%); MCV, mean corpuscular volume (fl); MCH, mean corpuscular haemoglobin (pg); MCHC, mean corpuscular haemoglobin concentration (g/l); RDWc, red cell distribution width (%); PLT, platelet count ($10^9$/l); PCT, platelet percentage; MPV, mean platelet volume (fl); PDWc, platelet distribution width (%).
C – control group, E1 (10 µg.kg$^{-1}$), E2 (100 µg.kg$^{-1}$), E3 (1000 µg.kg$^{-1}$) – experimental groups. Means with samesuperscripts within the same row differ significantly ($p < 0.05$). The values shown are the mean ± SD (standard deviation).

## CONCLUSION

In this experiment the chronic intramuscular application of quercetin and acute dose of T-2 toxin 72 hours before slaughteredresulted in slight changes in haematological parameters of rabbits. Administration of quercetin three times a week and T-2 toxin before slaughter significantly increased the level of MI% in E3, E4 and E5group in comparison with control group (E1).We found significant increase of MI% between E3*vs*.control group (C2). Also we found significant increase of MI% in E2, E3, E4, E5 and E6 in comparison with E1. Increase of MID% could be result of the higher doses quercetin treatment and T-2 toxin exposure. Also we found increase in MID, however without significant differences. We observed insignificant decrease in LY% in all experimental groups in comparison with the control group. Quercetin/T-2 toxin preparation caused increase in LYM in all experimental groups, but differences among the groups remained insignificant. Application of T-2 toxin insignificantly increased WBC in E1 and E3 groups in comparison with the control group.

Further investigation with different doses of quercetin will be worthy of further investigation.

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